Spectrophotometric Estimation of Cefpirome Sulphate in Pharmaceutical Formulations

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Three simple, accurate, rapid and sensitive methods (A, B and C) have been developed for the estimation of cefpirome sulphate in its pharmaceutical dosage form. The method A and B are based on diazotization of primary amine group of cefpirome with sodium nitrite and hydrochloric acid followed by coupling with α-naphthylamine (method A) and N-(1-napthyl)ethylene diamine hydrochloride (method B) to form pink coloured chromogen with a characteristic absorption maxima at 512 nm and purple coloured chromogen exhibiting maximum absorption at 565 nm, respectively. The method C is based on the formation of red coloured chromogen with ferric chloride and 1,10-phenanthroline, which shows absorption maximum at 510 nm. The absorbance-concentration plot is linear over the range of 5-40 mcg/mL for method A, 2.5-20 mcg/mL for method B and 2.5-40 mcg/mL for method C. Results of analysis for all the methods were validated statisti-cally and by recovery studies.

Key Words: UV-Visible spectrophotometry, Cefpirome sulphate, α-Naphthylamine, N-(1-napthyl)ethylene diamine hydrochloride, 1,10-Phenanthroline, Ferric chloride.

INTRODUCTION

Cefpirome sulphate[1] is chemically 3-[(2,3-cyclo-penteno-1-pyridinium) methyl]-7-[2-*syn*-methoxyimino-2-(2-aminothiazol-4-yl)acetamido]ceph-3-em-4-carboxylate. It is a fourth generation cephalosporin effective against gram-positive and gram-negative bacteria, including both aerobes and anaerobes. It is indicated for complicated upper and lower urinary tract infections, respiratory tract infections, skin and soft tissue infection and bacteraemia. Survey of literature reveals that the drug is determined by using HPLC[2-4] and few spectrophotometric method[5]. The present study describes simple, sensitive, accurate, rapid and economical spectrophotometric methods A, B and C for the estimation of cefpirome sulphate in its formulations.

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EXPERIMENTAL

Elico ultraviolet-visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

All the chemicals used were of analytical reagent grade, (i) sodium nitrite: 0.2 % in distilled water, (ii) hydrochloric acid: 1 M in distilled water, (iii) ammonium sulphamate: 0.5 % in distilled water, (iv) α-naphthylamine: 0.2 % in methanol, (v) N-(1-napthyl)ethylene diamine hydrochloride (BM reagent): 0.1 % in distilled water, (vi) 1,10-phenanthroline: 0.1 M in distilled water and (vii) ferric chloride (hexahydrate): 0.03 M in distilled water.

Standard stock solution was prepared by dissolving 100 mg of cefpirome sulphate in 100 mL of distilled water to get a concentration of 1000 mcg/mL. This was further diluted to get the working standard solution of 50 and 100 mcg/mL.

Assay procedure

Method A: Aliquots of standard drug solution of cefpirome sulphate 0.5-4.0 mL (100 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 1 mL of hydrochloric acid and 1 mL sodium nitrite were added, mixed and cooled in an ice-bath for 5 min, then 1 mL of ammonium sulphamate was added and the test tubes were kept at room temperature for 3 min for complete neutralization of the excess nitrous acid formed in the reaction. Then finally 1 mL of α-naphthylamine solution was added and mixed well. Immediately pink colour was developed. The volumes in each test tube were adjusted to 10 mL with distilled water. The absorbances of the solutions were measured at 512 nm against reagent blank and the calibration curve was plotted.

Similarly, the absorbance of the sample solution was measured and the amount of cefpirome sulphate was determined by referring to the calibration curve.

Method B: Aliquots of standard drug solution of cefpirome sulphate 0.5-4.0 mL (50 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 1 mL of hydrochloric acid and 1 mL sodium nitrite were added, mixed and cooled in an ice-bath for 5 min, then 1 mL of ammonium sulphamate was added and the test tubes were kept at room temperature for 3 min for complete neutralization of the excess nitrous acid formed in the reaction. Then finally 2 mL of BM reagent solution was added and mixed well. The volumes in each test tube were adjusted to 10 mL with distilled water.

The absorbance of the purple coloured solutions were measured at 565 nm against reagent blank, within 0.5 h and the calibration curve was plotted. Similarly, the absorbance of the sample solution was measured and the

amount of cefpirome sulphate was determined by referring to the calibration curve.

Method C: Aliquots of standard drug solution of cefpirome sulphate 0.25-4.0 mL (100 mcg/mL) were taken and transferred into series of graduated test tubes. To each test tube 2 mL of ferric chloride and 2 mL of 1,10-phenanthroline (0.1 M) were added. The test tubes were allowed to stand in water bath at 60 ºC for 15 min. The test tubes were then cooled to room temperature and the solutions were made upto 10 mL with distilled water. The absorbance of the red coloured chromogen was measured at 510 nm against reagent blank and a calibration curve was constructed. The absorbance of the sample solution was measured and the amount of cefpirome sulphate was determined by referring to the calibration curve.

The methods were extended for the determination of cefpirome sulphate in parenteral preparation (cefrom 1.0, Aventis) was choosen. The powder for injection contains sodium carbonate as excipients. A quantity of the powder equivalent to 100 mg was accurately weighed and transferred to 100 mL volumetric flask and made up to mark with distilled water. The contents of the flask were thoroughly mixed and filtered. Appropriate volume of the filtrate was suitably diluted to get a 100 mcg/mL concentration. The sample solution was analyzed as described, in the above-mentioned methods. The analysis procedure was repeated three times and the results of analysis are shown in Table-2.

Recovery studies: To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The percentage recoveries thus obtained were given in Table-2.

RESULTS AND DISCUSSION

In the present study, the method A and B are based on diazotisation of the primary amine group present in cefpirome using hydrochloric acid and sodium nitrite, followed by coupling with α-naphthylamine and BM reagent, respectively. The method C is based on the reduction of ferric chloride to ferrous form by the drug, which forms complex with 1,10-phenanthroline to yield red coloured chromogen. The conditions required for the formation of coloured complexes were optimized. Statistical analysis was carried out and the results of which were satisfactory. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1.

The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION DATA

Parameters		Method A	Method B	Method C
λ_{max} (nm)		512	565	510
Beer's law limits (mcg/mL)		5.0-40	2.5-20	2.5-40
Molar absorptivity (L/mol cm)		7.52×10^3	4.69×10^3	1.26×10^3
Sandell's sensitivity ($\mu g/cm^2/0.001$ absorbance unit)		0.6849	0.8928	0.4065
Regression equation* (Y)	Slope (m)	0.0010	0.0010	0.0020
	Intercept (c)	0.0501	0.0055	0.0268
Correlation coefficient (r)		0.9998	0.9989	0.9999
Precision (Relative Standard Deviation %)		0.1400	0.7740	0.2820
Standard error of mean		0.0185	0.0043	0.0170

*Y= mx + c, where x is the concentration in μg/mL and Y is absorbance unit

The reproducibility and precision of the methods are very good as shown by the low values of coefficient of variance (CV). Recovery studies were close to 100 % that indicates the accuracy and precision of the proposed methods and also indicates non-interferences from the formulation excipients. All the validated parameters are summarized in Table-2.

TABLE-2
ASSAY OF CEFPIROME SULPHATE IN PHARMACEUTICAL FORMULATION

Labeled amount (mg)	*Obtained (%) by proposed method			**Recovery by proposed method		
	A	B	C	A	B	C
1000	100.1	100.2	100.5	99.8	99.7	100.5
1000	99.9	100.1	100.8	99.9	99.6	100.3

*Average of three determinations; **After spiking the sample.

In conclusion, the proposed methods are simple, sensitive, accurate and economical for the routine estimation of cefpirome sulphate in bulk and in its formulations.

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